EFFECTS OF MICROGRAVITY ON GROWTH HORMONE CONCENTRATION AND DISTRIBUTION IN PLANTS

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ABSTRACT

On earth, gravity affects the distribution of the plant growth hormone, indole-3-acetic acid (IAA), in a manner such that the plant grows into a normal vertical orientation -- shoots up, roots down. How the plant controls the amount and distribution of IAA is only partially understood and is currently under investigation in this laboratory. The question to be answered in the flight experiment is: "How does gravity affect the concentration, turn over, and distribution of the growth hormone?" The answer to this question will aid in understanding the mechanism by which plants control the amount and distribution of growth hormone. Such knowledge of a plant's hormonal metabolism may aid in the growth of plants in space and will lead to agronomic advances.

INTRODUCTION

Ground-based studies:

The shoot of a young plant, placed in a horizontal position, grows back to a vertical orientation (Figure 1). The response begins within minutes after the plant is placed horizontally and vertical orientation is restored at a rate of $1^{o} \times min^{-1}$ (Bandurski et al, 1984). How the plant perceives gravity and how the gravity signal is transduced into an asymmetric growth response is only partially understood (Wilkins, 1984; Bandurski et al, 1986a). The plant's gravity response, and the lack of that response in micro-gravity, will be important in attempts to grow plants under micro-gravity conditions.

Gravity detection:

Owing to the pervasiveness of gravity, it is likely that plants sense gravity by more than one mechanism. For example, some plants may utilize the settling of dense starch grains, statoliths, to the bottom of the cell as a gravity-sensing mechanism (Bandurski et al, 1984; Sievers & Hensel, 1982). However, there is also evidence that a mutant plant, lacking phosphoglucomutase in its chloroplasts -- and thus lacking starch-filled statoliths -- can sense gravity almost as readily as normal plants (Caspar T, Sommerville C. 1988. Personal Communication).

This mutant is detecting gravity without dense starch grains. Statoliths may perceive gravity in some plants but they are obviously not the only mechanism for gravity perception. For example, a mechanism for gravity sensing, not involving the settling of dense particles, has been proposed (Bandurski et al, 1986a). In this mechanism, any distortion of the cells' shape

or of the microtubular structures in the cytoplasm of the cell could be used for gravity sensing.

Membrane depolarization:

Despite the uncertainties regarding gravity sensing, it is known that both gravity and light stimuli result in membrane depolarization. This phenomenon has been studied for more than 50 years (Wilkins, 1984; Dolk, 1933) and has recently been studied elegantly by Tanada (1983) and by Sievers and colleagues ([1]Bandurski et al. 1988. In Press). Membrane depolarization is the first detectable response of a plant to a gravitational stimulus, occurring within 8 sec after the stimulus is given ([1]Bandurski et al., 1988. In Press). It is the rapidity of the depolarization response and its induction by two such diverse stimuli as gravity and light which suggests that membrane depolarization is an integral part of the tropic response.

Hormone asymmetry:

The next detectable response following membrane depolarization is an asymmetric distribution of the plant growth hormone, indole-3-acetic acid (IAA) (Bandurski et al, 1984; Bandurski et al, 1986a; [1]Bandurski et al, 1988). The central focus of our research has been the question, "How does the plant transduce a membrane depolarization into an asymmetric distribution of IAA?" We believe that emphasis on the chemical asymmetry, rather than on the more complex issue of growth asymmetry, will facilitate attaining an understanding of the gravity response at a molecular level.

A working theory:

This laboratory has developed a working theory for the transduction of the gravity stimulus into an asymmetric distribution of IAA. We postulate that a change in the orientation of the plant with respect to the gravitational field induces a membrane depolarization as discussed above (Bandurski et al, 1986a; Tanada, 1983; Behrens et al, 1985; [1]Bandurski et al, 1988) Next, we postulate that membrane depolarization open and/or closes plasmodesmatal channels between the plants vascular tissue and the surrounding cortical and epidermal tissues. IAA, calcium, and other substances, can then flow selectively into the bottom side of a horizontal stem inducing a more rapid growth rate on the bottom side of the stem. The plant would then grow into its normal vertical orientation. Evidence for this theory is reviewed in references Bandurski et al (1986a) and [1]Bandurski et al (1988).

Growth asymmetry:

Growth is complex involving the regulated occurrence of perhaps thousands of reactions. However, in our experimental system, employing 5 day old seedlings of corn (Zea mays), growth is an arithmetic function of IAA

[1]Bandurski RS, Schulze A, Desrosiers M, Jensen P, Epel B, and Reinecke D. 1989. Relationship between stimuli, IAA, and growth. In: Plant Growth Substances. 1988. Pharis R, Rood R, Eds. In press

concentration. Thus, we confirm and extend the earlier concepts (Went & Thimann, 1937) that growth is controlled by IAA and that an IAA asymmetry will result in a growth asymmetry.

Summary of ground-based research:

The intent of this laboratory has been to attempt to link membrane depolarization to a chemical asymmetry within the plant. The chemical asymmetry could then result in a growth asymmetry such that the plant grows back into its normal orientation.

In summary, the sequence of events is believed to be: 1) sensing of the gravitational stimulus; 2) transduction of the stimulus into a membrane depolarization; 3) transduction of the membrane depolarization into a chemical asymmetry; and 4) transduction of the chemical asymmetry into asymmetric growth.

Flight program:

We do not have a theoretical basis for predicting the effect of microgravity on the growth hormone IAA other than our working theory. We know that at 1 g, IAA becomes asymmetrically distributed within a horizontally-placed plant. We believe this asymmetric distribution to be owing to selective movement of IAA from the vascular stele into the surrounding cortical tissues with more IAA coming from the lower side of the stele. The flight experiment will tell us whether the channels between stele and cortex are open or closed in the absence of the gravitational stimulus. This knowledge will be of value in understanding how plants regulate their endogenous IAA levels and may help in the growing of the plants in space.

RESULTS

Synopsis of the experimental protocol:

The plant seeds (kernels) are wrapped in filter paper, loaded into canisters and water added 12 h prior to launch. Two canisters and one LN_2 freezer are placed in each of two middeck lockers. The plants are allowed to grow for 108 h (total hydration plus growth time equals 120 h) at which time two of the canisters are permitted to grow until shuttle landing. Upon landing the two unfrozen canisters and the two prefrozen canisters are put into a 35 VHC, Taylor-Warton liquid nitrogen refrigerator. After several hrs, the frozen canisters are transferred to a dry-ice shipping container, loaded with solid CO_2 and sent to East Lansing for analytical studies. In East Lansing, the plants will be dissected into roots, seed, and shoot tissue and ground in aqueous acetone for extraction and determination of free and ester IAA.

Experimental design:

The plants must be grown in darkness, in microgravity, and frozen prior to landing. We have designed the plant growth container to minimize crew handling time and eliminate the possibility of plant material or moisture escaping into the mid-deck of the shuttle. Figure 6 shows a photograph of the canisters used for plant growth. There are two compartments to each

canister. Table I summarizes the weight, contents and dimensions of the canisters.

TABLE I

Canister length	335.0	m m
Canister diameter	82.0	m m
Canister weight	860.0	g
(There are 2 compartments per canister)		
14 Teflon sleeves (7.07g ea) X2=	19.08	g
28 filter papers (2.15g ea) X2=	121.0	g
(Two filter papers per kernel)		
14 kernels(0.197g ea) X2=	5.52	g
water(8 m/kernel) X2=	224.0	g
Total per canister	1408.52	g
One fully charged LN2	14,870.0	g

So 4 canisters would weigh 5634 g and 2 fully charged LN2's would weight 29740g for a total experiment weight of 35374 grams.

Gas exchange:

The canisters are vented through 4 light baffled holes to permit gas exchange with the air of the middeck locker. As can be seen in Figure 2, the venting is adequate to prevent the build-up of CO_2 and there is no benefit by adding an ethylene absorbent. Figure 3 shows that the venting is adequate to prevent depletion of oxygen and again there is no benefit by adding an ethylene absorbent.

We conclude that the canisters are adequately vented for the growth of 28 seedlings during 120 h growth period.

The assay for IAA:

An important part of both the ground-based studies and the flight program has been the development of a senstive and reliable assay procedure for IAA. Owing to the lability of IAA, its presence in low (10^{-8} M) concentration, and the presence of 10^{-3} M interfering phenylpropene acids, an internal standard must be employed. Colorimetric, fluorometric, and radioimmunoassays have proven useless (Pengelly & Bandurski, 1983; Cohen et al, 1987). The following assay has proven to be sensitive and accurate and provides proof that it is really IAA that is being measured. We originally synthesized 4,5,6,7-tetra deutero IAA as an internal standard (Magnus et al, 1980) but this has now been replaced by IAA labeled with 6 atoms of ^{13}C in the benzene ring portion of the indole nucleus (Cohen et al, 1986).

Extraction of IAA from the plant tissue:

The plants from the two canisters frozen in space will be separated into shoots, seeds, and roots, weights recorded and the plants then homogenized in sufficient acetone to make the final acetone concentration 70%. (All percentages are vol/vol.) The plants from the remaining two canisters will

be treated similarly and used as "controls" since they will have had, at least, 90 minutes of recovery time at one, or more, g. Ground controls will be similarly treated. The homogenates will be filtered, residues washed and weighed, and the volume of the aqueous acetone extracts determined. Two thirds of each extract will be used for the determination of free IAA and one third will be used for determination of esterified IAA. To each extract a known amount of ^{13}C IAA will be added in amounts such that the ^{13}C IAA will range between 1 to 10 times the plant IAA. In addition, about 540,000 DPM of 22.6 Ci/mmole tritiated IAA will be added. This amount (1,884 picograms of 5- ^{3}H-IAA is one mass unit heavier then the plant's IAA and further is only 9.4% of the IAA of a 1 g sample containing 20 ng of IAA per g) and so does not interfere with the assay but facilitates locating peaks on chromatograms. The aqueous extracts are concentrated in vacuo, made to 50% aqueous ethanol, applied to a 2 ml bed volume DEAE-acetate column and the volumn washed with 10 column volumes of 50% ethanol-water to remove non-anionic compounds. The column is then gradient eluted with 50 ml of 50% acetic acid in the mixing flask and 50 ml of 50% aqueous ethanol containing 5% acetic acid in the reservoir. IAA elutes at about 20 ml. The samples for determination of free plus ester IAA will have been treated similarly except that the samples will first be hydrolyzed with 1 M NaOH for 15 min at 22°C, then adjusted to pH 2.5, and the IAA extracted into ether, concentrated, taken up in 50% aqueous ethanol and treated as above.

The pooled IAA containing sample is reduced to near dryness (50 µl of capryl alcohol was added to prevent foaming and to prevent the sample from going to dryness) in vacuo, taken up in 200 µl of 50% aqueous ethanol and applied to a 4.8 mm X 250 cm C18 reverse phase HPLC column. Development is with 30% aqueous ethanol containing 0.1% acetic acid. The radioactive sample is collected at about 12 ml, dried in vacuo, taken up in 100 µl of methanol, methylated with 300 µl of ethereal diazomethane (Bandurski et al, 1986b), dried and taken up in 20 to 50 µl of acetonitrile for GC-MS.

Gas chromatography is on a 12.5 m 0.2 mm wall coated OV-17 column butt connected to 15 cm of 0.5 mm uncoated quartz pre-column and using direct on column injection. The GC-MS is the Hewlett-Packard 5890-5970 table top model. As shown in Figure 4, the chromatography is very good, and as shown in Figure 5, the ratio of amounts of material at masses 189 and 195 and 130 and 136 is easily determined. Mass 189 is the molecular ion of methyl IAA and 136 is the quinolinium ion of $6C^{13}$ IAA. The ratios of ions at 195/189 and 136/130 agree within 0.1% giving assurance that only pure IAA is being measured.

EXPECTED BENEFITS

As indicated above, there is no adequate theoretical basis for predicting the effects of gravity, or the lack of gravity, on biological systems. Mammalian systems, although of primary importance in terms of humans in space, appear terribly complicated and may be less suited than plants and microorganisms for attaining an understanding of gravity effects at the molecular level.

We believe that our system, utilizing 5 day old dark-grown corn plants is possibly the best eucaryotic plant system available. It is a closed system since, in darkness, the plants must obtain all of their nutrients and their growth hormone, IAA, from seed (Bandurski et al, 1986a; 1Bandurski et al, 1988; Bandruski et al, 1986b; Reinecke & Bandurski, 1987). Further, we have evidence that the targets for the gravitational response on earth, are the

plasmodesmatal channels connecting the vascular tissues of the stele with the cortical and epidermal tissues.

We have not completed our electrophysiological studies and so we can not predict whether the plasmodesmatal channels will be open or closed in microgravity. However, following the flight experiment we will be able to measure the size of the plants, their dry weight, how much IAA and IAA conjugates are in the shoot and, importantly, the amount of IAA and IAA conjugates left in the shoot. Such knowledge will provide another important set of facts which must be fit into any working theory for the molecular basis of the gravity effect on plants.

Such knowledge will be of practical value to terrestrial agriculture. Whether this knowledge will result in important advances in space technology is unknown. If there are no important microgravity effects, it will be of aid to the space station program to know there are no fundamental hormonal problems that prevent a successful agriculture in space. If there are microgravity effects than it is possible that a technology based upon substitution of electrical potentials for the gravitational stimulus might be of practical value in facilitating a space based agriculture (Desrosiers & Bandruski, 1988). Either result must ultimately fit into theories concerning how a plant regulates it endogenous hormone levels.

OBJECTIVE

We know that hormones control growth and development, but what controls the amount of the hormone? That is the ultimate objective of this experiment.

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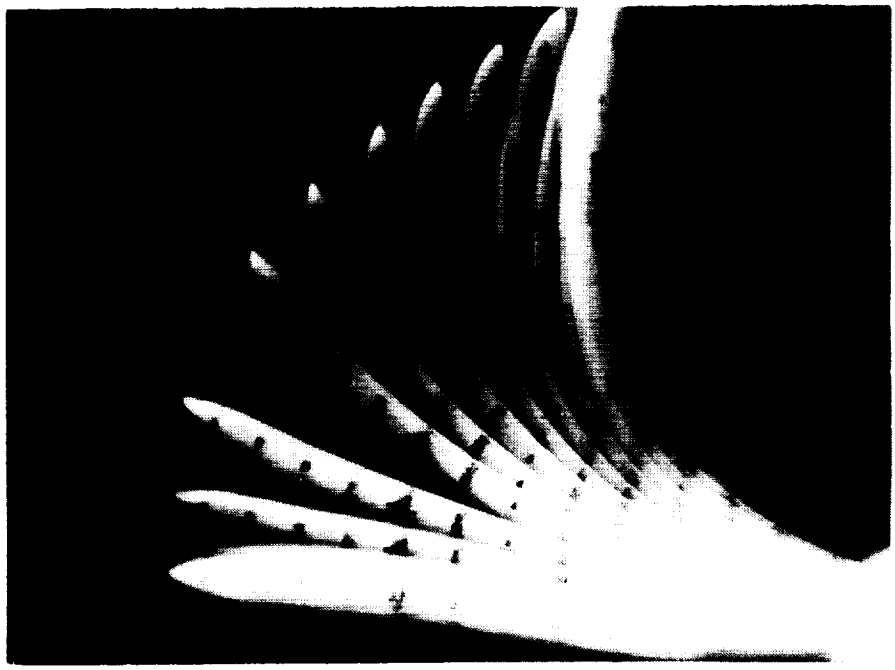

Fig. 1. Time lapse photograph of a seedling of *Z. mays* during gravitropic curvature. The intial photograph was taken just as the seedling was placed horizontally. Successive photographs are taken at 15-min intervals. The India-ink marked 'N' indicates the node between the coleoptile and mesocotyl..

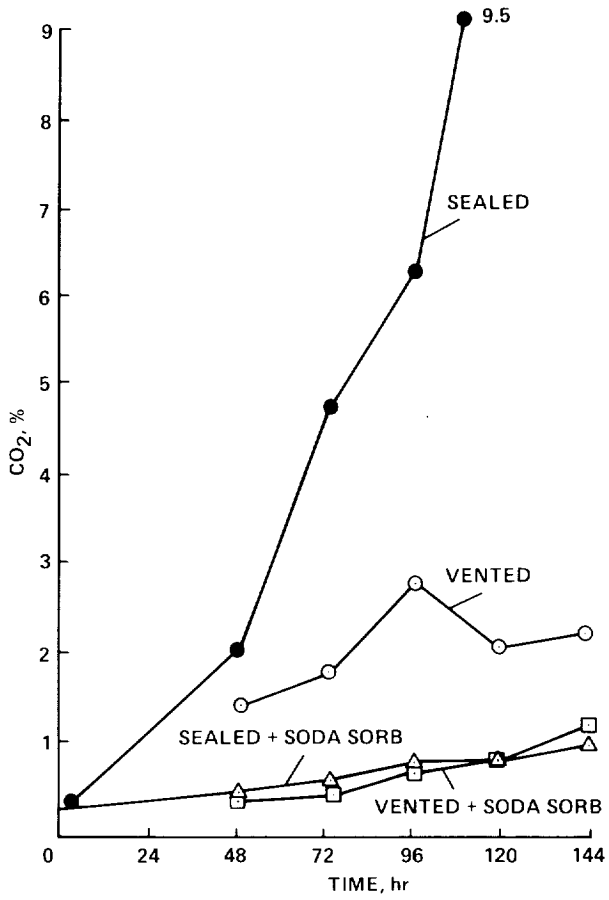

Fig. 2. Venting the canister prevents the build up of carbon dioxide so that a CO_2 absorbent such as soda sorb need not be added. Each compartment of the canister contained 14 germinating kernels of corn (Zea mays) for 120 h.

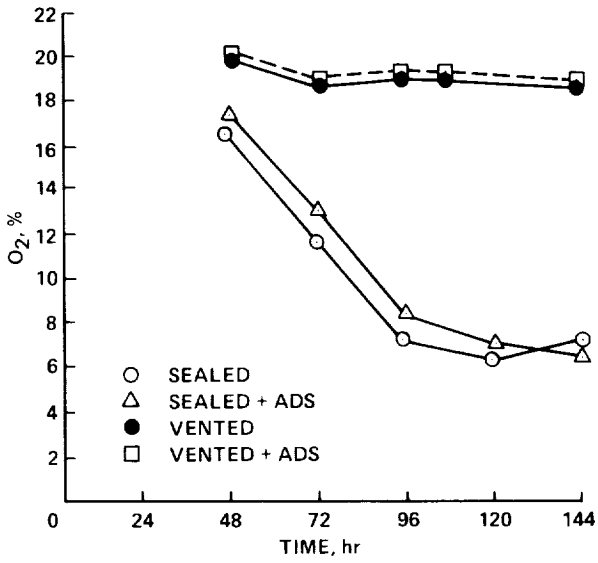

Fig. 3. Venting the canister prevents oxygen depletion. Addition of an ethylene and carbon dioxide absorbent did not change the per cent oxygen in the gas phase. Each compartment of the canister contained 14 germinating kernels of corn (Zea mays) for 120 h.

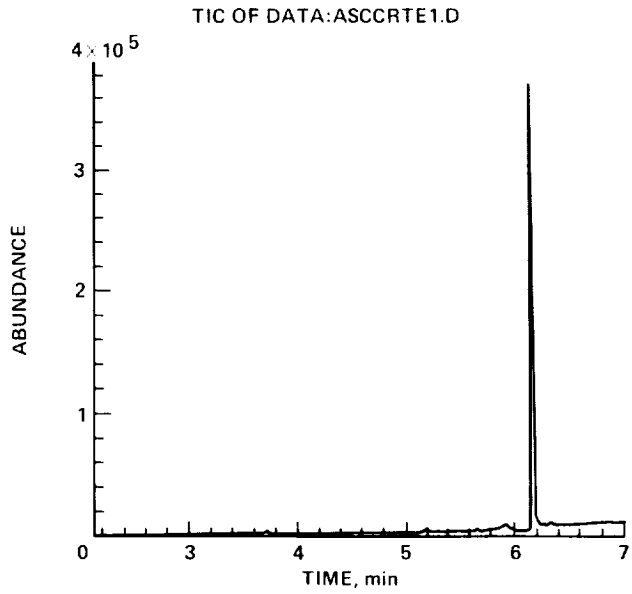

Fig. 4. Total ion current monitored as a function of retention time on a 12.5m OV-17 WCOT. As can be seen the purified and methylated IAA from the plant is almost free of any contaminants. This, possibly excessive, purification prior to GC/MS assay keep the injector and columns clean and improves our day to day sensitivity.

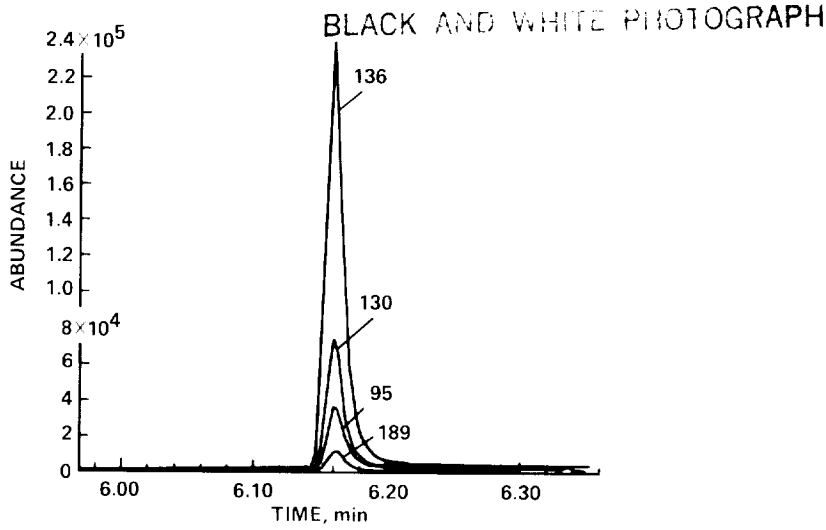

Fig. 5. Monitoring of massess 195 and 189, the molecular ions of the methyl ester of 6C13 IAA and plant IAA, and 136 and 130, the quinolinium ions of 6C13 IAA and plant IAA. Agreement of the ratio 195/189 and 136/130 is usually within 0.1% giving assurance that the compound being measured is, in fact, IAA.

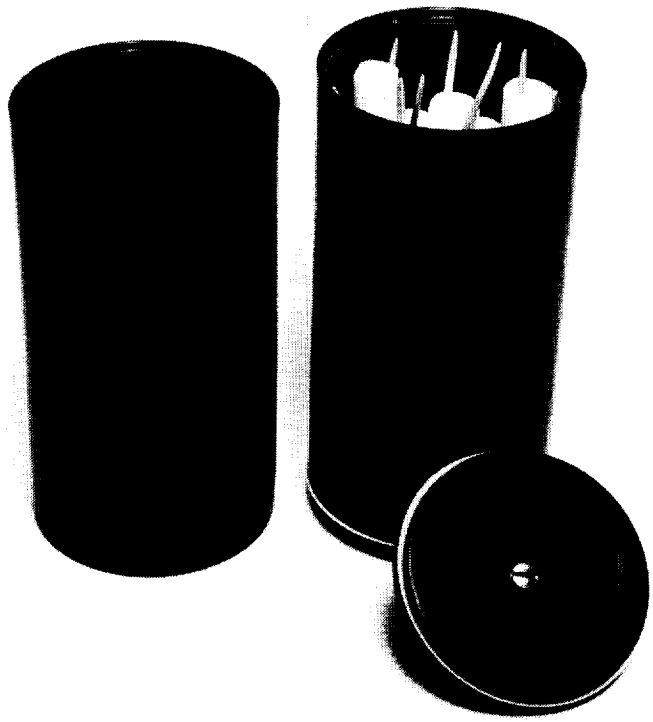

Fig. 6. A photograph of the plant canister separated into its two compartments. The lid screws into the top of one compartment which then screws into the bottom compartment to comprise the canister of two compartments. Construction is of anodized aluminum.